## REMARKS

### Claim Status

Claim 25 has been amended by incorporating the subject matters of claims 21 and 26. As amended, claim 25 is in an independent form.

Claims 21 and 26 have subsequently been cancelled.

Claim 27 has been amended to update dependency as a result of cancellation of claim 26.

New claim 28 has been added, which is directed to a pharmaceutical composition comprising the same kind of bivalent diabody as that of claim 21 with additional characterization of the diabody in feature (d) - it does not posses T cell activating properties in a PBMC culture -. Support for this new claim is clearly found in claims 21, 25 and 26, and support for feature (d) is clearly found in the last paragraph of page 27 of the instant specification.

Claims 22-24 have been cancelled, the subject matters of which have been rewritten into new claims 29-31 with updated dependency and preamble. That is, support for new claims 29-31 is clearly found in claims 22-24.

Previously withdrawn claims 14 and 17 have been amended with updated dependency as a result of the addition of new claim 28.

Applicants respectfully submit that the foregoing amendments do not introduce any new matter to the original application as filed. With the present amendments, claims 14, 16-17, 25 and 27-31 are pending. It is noted that new claims 28-31 fall within the scope of the previously elected invention. As such, they should be joined with claims 25 and 27 for further examination.

It is further noted that withdrawn claims 14 and 16-17 are patentably related to the elected claims as product and process of use. Applicants respectfully request that the withdrawn process of use claims be rejoined with the elected product claims and fully examined for patentability upon the allowance of the product claims. *See* MPEP §821.04(b).

### Claim Rejections - 35 U.S.C. § 103(a)

Claims 21, 23 and 25 remain rejected and previously added claims 26 and 27 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Smith et al. (WO 9847531; "Smith" hereafter) in view of Hsu et al. (Transplantation. 1999 Aug 27; 68(4):545-54; "Hsu" hereafter), Holliger et al. (U.S. Pat. No. 5,837,242; "Holliger" hereafter) and Chapman et

al. (Nat. Biotechnol. 1999 Aug; 17(8):780-3; "Chapman" hereafter). In response, Applicants respectfully traverse this rejection.

It is first noted that claims 21, 23 and 26 have been cancelled without prejudice. New claims 28 and 30 have been added incorporating the subject matters of claims 21, 23 and 26. Applicants extend the present rejection to newly added claims 28 and 30.

*Present invention as claimed*

The instantly claimed invention relates to a pharmaceutical composition comprising an anti-CD3 antibody of the so-called diabody format which (a) is devoid of constant antibody domains; (b) contains variable domains specific to human CD3; and (c) is capable of suppressing an immune reaction.

Particularly, the present invention provides a less immunogenic antibody which prevents T cell activation (*see* p. 2, ¶ 2-3 and p. 8, last ¶ of the specification). The diabody of the present invention does not possess T cell activating properties (*see* Figure 13), while immunosuppressive properties are retained (*see* Figure 14). The complete lack of mitogenic and lytic activity by the anti-CD3 diabody had not been previously demonstrated for any other anti-CD3 molecule including $F(ab')_2$ antibody fragments. These remarkable properties of the anti-CD3 diabody of the present invention could not be expected from the prior art as will be further explained below.

*Surprising and unexpected immunosuppression effect of anti-CD3 diabody*

It is surprising and unexpected in view of the prior art that the anti-CD3 diabody of the present invention lacks any mitogenic and lytic activity.

Smith teaches a selective T cell activation by FcR-nonbinding anti-CD3 IgG antibodies (e.g., p. 4, ¶ 1). With respect to $F(ab')_2$ anti-CD3 antibody, Smith teaches that $F(ab')_2$ fragments exhibited significantly reduced T cell activation and fewer side effects. The reduction in T cell activation by $F(ab')_2$ fragments is confirmed by Woodle et al. (Transplantation. 1991 Aug; 52(2):354-60; "Woodle" hereafter, cited by the Examiner), which further teaches that the observed T cell proliferation is induced by bivalent TCR crosslinking: "*rigorously purified $F(ab')_2$ preparations demonstrated minimal T cell activation, suggesting TCR and macrophage FcR crosslinking as necessary*" (abstract, ll. 21-23) and "*soluble $F(ab')_2$ fragments induced*

*minimal proliferation, suggesting that bivalent TCR crosslinking alone induced T cell activation*"(p. 356, ¶ 3, ll. 8-10).

Thus, both Smith and Woodle teach that bivalent CD3 binding induces partial T cell activation and proliferation. Moreover, Woodle teaches that $F(ab')_2$ antibodies are still capable of mediating FcR cross-linking, although they are devoid of the antibody constant regions. As such, one skilled in the art would not have had a reasonable expectation of success that a diabody which is devoid of the antibody constant domains does not induce any T cell activation and proliferation. Hence, it is surprising and non-obvious that the bivalently binding anti-CD3 diabody of the present invention demonstrates a complete lack of lytic activity (*see* Figure 9 of the present application) while retaining the immunosupressive properties.

Further, Holliger expressly teaches that "diabodies may also bind simultaneously to two epitopes on the same surface... by crosslinking the CD3 antigen so as to activate T-cells" (col. 22, ll. 13-17). That is, Holliger also teaches T cell activation by an antibody devoid of constant regions.

"T cell activation" is generally considered in the art as a stimulation of T lymphocytes to undergo mitosis, and increased lymphokine production and cytotoxic cell activity (*see* Smith, p. 24, l. 24 thru p. 26, l. 9). On the other hand, the intended "immunosuppression" effect according to the present invention relates to the prevention of T lymphocyte activation (*see* p. 9, ¶ 3 of the specification). Thus, the T cell activation mentioned by Holliger is exactly the opposite of the intended effect of the present invention.

Because the generally accepted meaning of T cell activation is lytic and mitogenic activities induced by stimulated T cell receptors, one skilled in the art would understand Holliger's reference to T-cell activation as meaning that their propensity for cytolytic or mitogenic activity would be stimulated by diabodies. Holliger does not teach or suggest whether diabodies can inhibit T cell proliferation. Also the lack of antibody constant regions in the diabodies does not indicate that immunogenic reactions can be avoided, as Woodle teaches that $F(ab')_2$ antibodies devoid of FcR binding constant regions can still induce some T cell activation by TCR cross-linking. Therefore, one skilled in the art would not have had a reasonable expectation of success that an anti-CD3 diabody lacking lytic and mitogenic activity can be made. Considering the T cell activating function of diabodies suggested by Holliger, one skilled

in the art, when faced with the problem of making low mitogenic antibodies, would not have had any incentive to replace Smith's anti-CD3 $F(ab')_2$ with an anti-CD3 diabody.

In the present Office Action, the Examiner puts forth that the teachings of Holliger and Smith would be consistent (p. 4, ¶ 3). However, the immunomodulation of T cells by the non-Fc binding anti CD3-IgG3 antibody to which the Examiner is referring is not the general suppression of T cell activation due to down-modulation (or disappearance) of the TcR. Smith teaches that the antibody which has low affinity FcR binding activity delivers a partial T cell signal which promotes Th2 cell proliferation and suppression of Th1 cell responses (e.g., p. 18, ll. 23-28) and as such, its action may be fairly complex. That is, Smith teaches a selective T cell activation that contributes to the immunosuppressive activity (e.g., p. 4, ¶ 1), which immunomodulation has to be distinguished from the general immunosuppression by inducing the internalisation and disappearance of the TcR as shown by the anti-CD3 diabodies according to the present invention (*see* Example 9). As such, the possibility of T cell activation mentioned by Holliger points away from suppressing T cell activation by inducing the internalisation and disappearance of the TcR. In fact, Holliger does not suggest such immunosuppressive properties. Holliger's teaching that cross-linking the CD3 antigen activates T-cell would not be considered by one skilled in the art as a function consistent with suppressing T cell activation by inducing internalisation and disappearance of the TcR that is intended by the present invention.

None of the cited prior art references teaches or suggests that an anti-CD3 antibody lacking lytic and mitogenic activity can be obtained. In contrast, Smith and Woodle both suggest that immunosuppression is accompanied by some T cell activation. Moreover, none of the cited references teaches or suggests that an anti-CD3 diabody shows a low or less mitogenic activity. In contrast, Holliger clearly assigns T cell activating properties to a diabody. Therefore, one skilled in the art, when faced with the problem of making less mitogenic anti-CD3 antibodies, would not have made, with a reasonable expectation of success, an anti-CD3 diabody that does not possess T cell activating properties but efficiently induces immunosuppression. As such, it is not obvious to select an anti-CD3 diabody for making a pharmaceutical composition for immunosuppression.

Moreover, the suppressive activity of the diabody according to the present invention was unexpected. In the present Office Action, the Examiner states that much of Applicants' previous

assertions in this respect are unclear; in particular, the comparison of a diabody linking T cells and a diabody linking a T cell with a B cell. Applicants provide the following clarifications.

At the filing date of the present application, it was known that diabodies could activate T cells by cross-linking of the TcR. For example, Kipriyanov et al. (Int. J. Cancer, 1998, 77:763-771; "Kipriyanov I" hereafter; note this reference was previously submitted with an Information Disclosure Statement on October 7, 2005 as reference No. 22; copy is again enclosed herewith for the Examiner's convenience) clearly shows that a bispecific anti-CD3 x anti-CD19 diabody is able to efficiently activate the T cells and cause lysis of the CD19 presenting B lymphocytes. The cross-linking of the TcR is mediated by the multiple anchorage sites of the diabody on the B cell surface analogous to the binding of antibody Fc domains to FctR-bearing cells in the T-cell activation process. Replacing the anti-CD19 moiety with an anti-CD3 moiety, resulting in a bivalent anti-CD3 diabody and providing a T-cell instead of the B-cell for providing the multiple anchorage sites needed to cross-link the TcR, might be expected to provide approximately the same amount of activation, possibly more, since both of the cross-linked cells are T cells. Surprisingly and unexpected, however, the T-cells are not activated by a bivalent anti-CD3 diabody and the TcR disappears from the cell surface.

Moreover, the immunosuppressive activity of bivalent anti CD3 $F(ab')_2$ fragments was not prognostic for the unexpected suppressive activity of the claimed diabody because of their different geometries. The binding sites of the non Fc-binding IgG3 antibody and the $F(ab')_2$ fragments are generally pointed in approximately the same direction, a favorable position for binding two antigens on the same cell surface (e.g., *see* Figs. 1A and 1L of Holliger). In contrast, the antigen binding sites of a diabody point in opposite directions which differ from that in an antibody or Ig superfamily structure (*see* Holliger, col. 15, ll. 49-58) and, therefore, is a favourable position for binding antigens on two different cells, e.g. two T cells as discussed in the immediate preceding paragraph. Because of the rigid structure of diabodies with binding sites pointing in opposite directions – in contrast to the flexibility of the V-shaped structure of IgG antibodies and their $F(ab')_2$ fragments permitting their binding sites to turn towards antigen epitopes on the cell surface – one skilled in the art could not expect that a diabody is capable of efficiently binding to CD3 such that internalisation and disappearance of the TcR is induced. Therefore, it is not obvious that the anti-CD3 diabody according to the present invention down-

modulates CD3 with a similar efficacy as the anti-CD3 OKT3 IgG (*see* Fig. 14 of the present application). In particular, this would not have been expected from the prior art as noted by Holliger or Kipriyanov, which indicates that such diabody molecules would activate T cells.

*Unexpected and superior immunosuppression effect of diabody*

At pages 5-6 of the present Office Action, the Examiner finds Applicants' previously submitted Declaration by Dr. Melvin Little unconvincing, stating that Applicants failed to compare the claimed invention to the nearest prior art which could be any divalent OKT3 derived anti-CD3 antibody lacking an Fc domain, such as OKT3 derived $F(ab')_2$ or bivalent $(Fab'zipper)_2$.

Applicants herewith submit a new Rule 132 declaration by Dr. Melvin Little, one of the co-inventors of the present application, stating the superior and unexpected immunosuppression property of the instantly claimed diabody. Therein, the PBMC proliferative effect of OKT3 diabodies as claimed in the present invention is compared to the proliferative effect of OKT3 derived $F(ab')_2$ according to Smith and Woodle.

The data show that the anti-CD3 OKT3 diabody according to the present invention did not induce any proliferation, while the OKT3 $F(ab')_2$ fragments showed an increased stimulation of PBMC proliferation at antibody concentrations of above 1 μg/ml. It is noted that purified $F(ab')_2$ fragments without contamination as shown in the HPLC size exclusion chromatography diagrams of Figs. 1 and 2 were tested and the results concerning the OKT3 $F(ab')_2$ are in compliance with the data of Woodle, which reports a similar significant stimulation of PBMC proliferation by OKT3 $F(ab')_2$ at an antibody concentration of 10 μg/ml (*see* Table 1 thereof).

At page 6 of present Office Action, the Examiner refers to the results of Woodle and points to the effects of highly purified $F(ab')_2$ on the proliferation of human PBMCs at a concentration of $10^3$ ng/ml. Such concentration is believed to be corresponding to a concentration of $10^4$ pM, not $10^5$ pM given by the Examiner. The Examiner contends that the amount of proliferation at this concentration is comparable to that seen when using the anti CD3 diabody in Applicants' previously submitted Rule 132 declaration. Applicant cannot agree for the reasons stated below.

(1) The diabody, in contrast to the $F(ab')_2$ fragment, has never shown any effect on

proliferation at the concentration mentioned by the Examiner or at any other concentration that was used.

(2) In fact, the data of Woodle clearly demonstrate a large increase in the amount of proliferation at a concentration of 10 μg/ml (*see* Fig. 4 thereof) and small amounts of this fragment were shown to significantly potentiate the effect of the full-length anti CD3 antibody at very low concentrations. The amount of proliferation of the PBMCs at 10 μg/ml F(ab′)2 is approximately 43% of the amount of proliferation shown for the full-length anti CD3 OKT3 antibody at the same concentration. In contrast, it is shown in Fig. 13 of the present application that the diabody ($ScFv_6$) does not exhibit cell proliferation activities at a concentration of 10 μg/ml.

In conclusion, the presently submitted Rule 132 declaration demonstrates that anti-CD3 $F(ab')_2$ fragments, whether they were tested by Woodle or in the experiment presented in the declaration, provide a significant stimulation of PBMC proliferation at concentrations where no proliferation is observed for the same quantity of the instantly claimed diabody. Such lack of mitogenic and lytic activity of the claimed diabody is of a significant, practical advantage, e.g. administration of the claimed pharmaceutical composition will be much safer because the antibody of the present invention does not induce cell proliferation even at concentrations that are necessarily higher to achieve long term down-regulation of TCR.

In view of the foregoing amendments and remarks, Applicants respectfully submit that the cited references do not render the instant claims obvious. As such, the rejection under 35 U.S.C. §103(a) should be withdrawn.

Claims 22 and 24 remain rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Smith, in view of Hsu, Holliger and Chapman as applied to claims 21, 23 and 25 above, and further in view of Kipriyanov et al. (Protein Eng. 1997 Apr; 10(4):445-53; "Kipriyanov II" hereafter). In response, Applicants respectfully traverse this rejection.

It is first noted that claims 22 and 24 have been cancelled without prejudice, the subject matters of which have been rewritten into new claims 29 and 31. Applicants extend the present rejection to newly added claims 29 and 31.

Next, since claims 29 and 31 are dependent from claim 25 or 28, the limitations of

independent claims 25 and 28 are carried over to the dependent claims. As discussed above, Smith, Hsu, Holliger and Chapman, alone or combined, does not disclose or suggest an anti-CD3 diabody or a pharmaceutical composition comprising the diabody as claimed in claims 25 or 28.

Kipriyanov does not disclose or suggest that an OKT3 scFv shows an immunosuppressive effect. Therefore, the addition of Kipriyanov does not cure the deficiency of Smith, Hsu, Holliger and Chapman as discussed above.

Additionally, one skilled in the art would not have been motivated by Kipriyanov to use the specific linker of claim 29 or to use the modified domain of claim 31 to make an anti-CD3 diabody. In fact, Kipriyanov II describes an OKT3 scFv containing the 17 amino acid peptide linker "AKTTPKLEEGEFSEARY". Holliger teaches that "the linker may consist of 10 or more amino acids" and "the linker may be 15 amino acids or longer" (col. 3, ll. 57-61). Therefore, both Kipriyanov II and Holliger point to a peptide linker that is longer than the 6-residue linker recited in claim 29. As such, one skilled in the art, when combining the teachings of Holliger and Kipriyanov II, would be motivated to make a diabody with a peptide linker that is at least 10 amino acids, preferably, with the 17 amino acid peptide linker as taught by Kipriyanov II. Clearly, neither reference suggests a peptide linker as set forth in SEQ ID NO:1 of the present invention.

Moreover, Kipriyanov II does not teach or suggest that the reported OKT3 scFv antibody has immunosuppressive activity. Because, neither Holliger nor Kipriyanov II suggests that an OKT3 based antibody has an immunosuppressive activity, one skilled in the art would not have had a reasonable expectation of success that a pharmaceutical composition comprising an OKT3 diabody is immunosuppressive. Therefore, it would not have been obvious to make the serine mutation to obtain an OKT3 diabody for the formulation of the pharmaceutical composition of claim 31.

In view of the foregoing amendments and remarks, Applicants respectfully submit that the cited references do not render the instant claims obvious. As such, the rejection under 35 U.S.C. §103(a) should be withdrawn.

**New Ground of Rejection: Claim Rejection - 35 U.S.C. § 102(b)**

Claim 21 stands rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Holliger. In response, Applicants respectfully traverse this rejection.

It is noted that claim 21 has been cancelled without prejudice, the subject matter of which has been incorporated into amended claim 25. Applicants extend the present rejection to claim 25.

Claim 25 is directed to a pharmaceutical composition comprising a bivalent diabody and a suitable pharmaceutical carrier for immunosuppression effects. Holliger does not disclose a pharmaceutical composition for immunosuppression effects. Therefore, Holliger does not anticipate claim 25.

In view of the foregoing amendments and remarks, Applicants respectfully request that the rejection of claim 21 under 35 U.S.C. § 102(b) be withdrawn.

**New Ground of Rejection: Claim Rejection - 35 U.S.C. § 103(a)**

Claims 21 and 25-27 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Holliger. In response, Applicants respectfully traverse this rejection.

It is first noted that claims 21 and 26 have been cancelled without prejudice, the subject matters of which have been incorporated into claim 25. Applicants extend the present rejection to newly added claim 28 as well.

Holliger teaches pharmaceuticals comprising polypeptides or multimers (*see* col. 8, ll. 31-32), wherein trimerisation of the polypeptides is feasible and the terms are used in relation to both multivalent and multispecific multimers (*see* col. 3, ll. 7-13). Holliger does not suggest that a bivalent, dimeric anti-CD3 diabody is immunosuppressive and prevents T cell activation. Instead, Holliger teaches that cross-linking of CD3 activates T cells. Therefore, one skilled in the art would not have had a reasonable expectation of success from the teaching of Holliger that a pharmaceutical composition comprising a bivalent, dimeric anti-CD3 diabody can be used for immunosuppression.

In view of foregoing amendments and remarks, Holliger does not render the instant claims obvious.

**Conclusion**

In light of the foregoing amendments and remarks, Applicants respectfully submit that the instant claims are in condition for allowance and that the previously non-elected process of use claims should be rejoined for further examination upon allowance of the elected product claims. *See* MPEP §821.04(b).

Respectfully submitted,

/j. wendy davis/

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